Morphology-dependent bactericidal activities of Ag/CeO₂ catalysts against *Escherichia coli*

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ABSTRACT

Silver-loaded CeO₂ nanomaterials (Ag/CeO₂) including Ag/CeO₂ nanorods, nanocubes, nanoparticles were prepared with hydrothermal and impregnation methods. Catalytic inactivation of *Escherichia coli* with Ag/CeO₂ catalysts through the formation of reactive oxygen species (ROS) was investigated. For comparison purposes, the bactericidal activities of CeO₂ nanorods, nanocubes and nanoparticles were also studied. There was a 3–4 log order improvement in the inactivation of *E. coli* with Ag/CeO₂ catalysts compared with CeO₂ catalysts. Temperature-programmed reduction of H₂ showed that Ag/CeO₂ catalysts had higher catalytic oxidation ability than CeO₂ catalysts, which was the reason for that Ag/CeO₂ catalysts exhibited stronger bactericidal activities than CeO₂ catalysts. Further, the bactericidal activities of CeO₂ and Ag/CeO₂ depend on their shapes. Results of 5,5-dimethyl-1-pyrroline-*N*-oxide spin-trapping measurements by electron spin resonance and addition of catalase as a scavenger indicated the formation of •OH, •O₂[−], and H₂O₂, which caused the obvious bactericidal activity of catalysts. The stronger chemical bond between Ag and CeO₂ nanorods led to lower Ag⁺ elution concentrations. The toxicity of Ag⁺ eluted from the catalysts did not play an important role during the bactericidal process. Experimental results also indicated that Ag/CeO₂ induced the production of intracellular ROS and disruption of the cell wall and cell membrane. A possible production mechanism of ROS and bactericidal mechanism of catalytic oxidation were proposed.

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1. Introduction

The unique properties of metal nanoparticles have a great potential in research and diverse applications [1–3]. These properties include chemical, mechanical, electrical and optical characteristics as well as catalytic and biological activities. The antimicrobial properties of nano-scale metal and metal oxide particles such as Ag, TiO₂, ZnO, and MgO have been the focus of research and application in antimicrobial coatings. Such metal nanoparticles interact with microbial cells through multiple biochemical pathways, for instance, via the production of reactive oxygen species (ROS) such as •OH, H₂O₂, and •O₂[−], which can damage cell structures and ultimately cause cell death [4–8]. Generally, photocatalysts such as TiO₂ can effectively produce ROS when applied in water [9,10]. However, photocatalysis technology requires the use of photon energy and complex devices. Therefore, the development of non-photocatalysis procedures containing abundant ROS formation is necessary for disinfection. According to the literature, many inorganic bactericidal materials such as MgO, CaO, and silver loaded materials can inactivate microorganisms through catalytic oxidation processes involving ROS [4,6,11–16]. For pure oxides such as MgO and CaO, the bactericidal activity is low [12]. Although the bactericidal activity of

silver-loaded materials is high, Ag⁺ elution is an issue [16]. To improve bactericidal activity and suppress the elution of Ag⁺, it is necessary to develop a new inorganic material to effectively produce ROS and reduce Ag⁺ elution.

Due to the high oxygen transport and storage capacities of ceria (CeO₂), notable surface oxygen species form on CeO₂ nanomaterials [17] widely employed in heterogeneous catalysis [18–20]. Ceria is an interesting oxide because oxygen vacancy defects can be rapidly formed, thus O vacancies and ROS in CeO₂ are naturally anticipated in catalytic processes. Since ROS play an important role in the catalytic bactericidal process [14–16], CeO₂ is a potential bactericidal material through ROS formation either as a catalyst or catalyst support, and thus it is important to investigate its bactericidal activity. The toxicity effects of CeO₂ nanoparticles on bacteria have been studied recently [21,22]. However, little research has been conducted on the role of ROS and related bactericidal activity of CeO₂. Few reports are available on the effect of CeO₂ shape on bactericidal activity although the catalytic activity of CeO₂ is usually related to its shape and size [23,24]. Furthermore, considering the high catalytic oxidation ability of Ag/CeO₂, strong interaction of Ag with CeO₂, and unusual sinter resistance [25,26], Ag/CeO₂ catalysts were prepared based on as-prepared CeO₂ to effectively decrease *Escherichia coli* survival through catalytic oxidation at room temperature and to decrease Ag⁺ elution depending on strong interaction. Hereby, bactericidal activities of CeO₂ nanorods, nanocubes, and

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nanoparticles prepared by facile hydrothermal synthesis and precipitation methods, and CeO₂ supported silver (Ag/CeO₂) catalysts were tested in this study. The reasons for different bactericidal activities of CeO₂ correlated with different shapes and for largely improved bactericidal activity of Ag/CeO₂ were explored. In addition, the formation of ROS was confirmed and the catalytic bactericidal mechanism was proposed.

2. Materials and methods

2.1. Preparation of catalysts

The CeO₂ nanorods and nanocubes were synthesized by a solution-based hydrothermal method, whereby Ce(NO₃)₃·6H₂O (3.0 g, AR grade, Tianjin Fuchen Chemical Reagent Factory, China) was dissolved in deionized water, and then mixed with proper amounts of 10 and 1 mol/L NaOH solution in a 100-mL Teflon bottle, respectively. The Teflon bottle was then placed in a stainless steel autoclave heated at 100 °C for 12 h. The CeO₂ nanoparticles were prepared by traditional precipitation, whereby Ce(NO₃)₃·6H₂O was dissolved in deionized water, and the pH of the solution was rapidly adjusted to 12 using 1 mol/L NaOH solution with stirring. The precipitate was centrifuged after hydrothermal treatment or precipitation, and the fresh precipitates were then separated by centrifugation and thoroughly washed with deionized water until the eluent became neutral. The obtained solid was dried at 60 °C for 24 h and calcined at 350 °C for 4 h in air.

The Ag/CeO₂ sample was prepared by impregnation through dispersing an appropriate amount of CeO₂ powder in an aqueous AgNO₃ solution. The mixed solution was stirred for 2 h at room temperature, followed by evaporation to dryness in a rotary evaporator at 60 °C under reduced pressure. The obtained solid was dried at 60 °C overnight and calcined at 550 °C for 3 h in air. The actual Ag content of Ag/CeO₂ product was detected using inductively coupled plasma optical emission spectrometer (ICP-OES) on an Optima 2000 (Perkin-Elmer Co.). In detail, 10 mg Ag/CeO₂ sample was dissolved with 5 mL concentrated HNO₃ (65%) and concentrated H₂O₂ (30%) with a volume ratio of 4:1. Then, the solution was diluted to 50 mL, followed by ICP-OES measurement.

2.2. Characterization

Powder X-ray diffraction (XRD) patterns were obtained on a PANalytical X'Pert PRO X-ray diffractometer (Japan) using Cu K α radiation ($\lambda = 0.154$ nm) at a scan rate of 6° (2 θ) min⁻¹, and were used to identify the phase constitutions in the samples.

The CeO₂ images were obtained using a Hitachi H-7500 electron microscope (Tokyo, Japan) with an operating voltage of 80 kV or a JEOL JEM-2011 or a Tecnai G2 20 high-resolution transmission electron microscope (HRTEM) with an acceleration voltage of 200 kV. The existence of silver was confirmed by energy dispersive spectroscopy (EDS).

Electron spin resonance (ESR) spectra were obtained using a Bruker model ESP 300E ESR spectrometer. The settings for the ESR spectrometer were center field 3480.00 G, microwave frequency 9.75 GHz, and power 20.15 mW.

Temperature-programmed reduction of H₂ (H₂-TPR) was also performed using quadrupole mass spectrometry to record the signals of H₂ ($m/z = 2$). Prior to TPR experiments, the samples (100 mg) were pretreated at 300 °C in a flow of 20 vol.% O₂/Ar (50 mL/min) for 1 h and cooled to room temperature. The samples were then exposed to a flow of 5 vol.% H₂/Ar (30 mL/min) at 30 °C for 1 h, followed by raising the temperature to 800 °C at a rate of 10 °C/min.

X-ray photoelectron spectroscopy (XPS) measurements were performed on a Thermo ESCALAB 250 spectrometer (Vacuum Generators, USA) using Al K α radiation (1486.6 eV) with a constant pass energy of 20 eV. The spectra were corrected by referencing C1s measurements at 284.8 eV.

2.3. Culture of *E. coli*

The *E. coli* ATCC 8099 bacterial strain was inoculated into lactose broth (LB) (Fluka Co. 61748, Switzerland) and cultured aerobically for 24 h at 3 °C with constant agitation. Aliquots of the culture were inoculated into fresh medium and incubated at 37 °C for 12 h until they reached the exponential growth phase. Bacterial cells were collected using centrifugation at 8000 rpm for 10 min, with the pellet then washed and resuspended with sterilized water. Finally, the bacterial cells were diluted with sterilized water and immediately plated on LB agar plates. The colonies were counted after incubation at 37 °C for 24 h. Cell density corresponding to 10⁹–10¹⁰ colony forming units per milliliter (CFU/mL) was then achieved.

2.4. Test of bactericidal activity

One milliliter of *E. coli* suspension was injected into 99 mL of sterilized water, and the as prepared CeO₂ and Ag/CeO₂ were then added to the system. The final catalyst concentration was adjusted to an appropriate value, and the final cell concentration was 10⁷–10⁸ CFU/mL. The reaction mixture was stirred with a magnetic stirrer to prevent settling of catalyst. All materials used in the experiments were autoclaved at 121 °C for 20 min to ensure sterility. Bacterial suspension without the catalyst was used as the control. At time intervals of 10, 30, 60, and 120 min after the addition of the catalyst, 0.5 mL of bacterial suspension was withdrawn and immediately diluted 10-fold in series with 4.5 mL of 0.9% saline solution and plated on LB agar (Fluka Co. 61746) plates. Viable cell counts were determined visually as the number of colonies per plate in serial 10-fold dilutions after incubation at 37 °C for 24 h. The reaction temperature was maintained at 25 °C. All experiments were repeated in triplicate.

2.5. Reactive oxygen species detection

To determine the production of intracellular ROS, 2',7'-dichlorofluorescein-diacetate (DCFH-DA, Sigma) was used [27]. The *E. coli* samples were collected after centrifugation from LB agar and washed with phosphate-buffered saline (PBS) solution. The *E. coli* samples were then stained with 10 μ M DCFH-DA for 30 min. After that, *E. coli* samples were treated with Ag/CeO₂ in water. Cells stained with DCFH-DA served as the negative control, and H₂O₂ was used as the positive control. Relative fluorescence intensity was recorded using a fluorescent plate reader (Thermo) at an excitation wavelength of 485 nm and emission was measured at a wavelength of 530 nm. Fluorescence intensity was assayed, which was proportional to intracellular ROS concentration. The formation of highly fluorescent DCF was also estimated with a fluorescent microscope (Zeiss Scope A1).

2.6. Analysis of morphological and structural change

The transmission electron microscopy (TEM) measurements were used to provide insight into the size, structure, and morphology of *E. coli*. To avoid possible damage caused by specimen preparation, native *E. coli* or a suspension of a treated sample was fixed with 2.5% glutaraldehyde, dehydrated by successive soakings in 50%, 70%, 90% and 100% ethanol, and dropped onto copper grids with perforated carbon film. The samples were allowed to dry in air at ambient temperature and were examined using a Hitachi H-7500 electron microscope (Tokyo, Japan) operated at a 80 kV accelerating voltage.

Propidium iodide (PI) was used to examine the disruption of cellular membrane because PI can only influx into cells with disrupted membranes. The staining protocol was as proposed by the manufacturer. Bacteria were first treated with Ag/CeO₂ in water. The substrates were then washed with PBS and stained with PI dye and subsequently analyzed with a fluorescent microscope (Zeiss Scope A1).

2.7. Quantitative analysis of silver ions

To investigate the Ag^+ concentration eluted from the Ag/CeO_2 in ultrapure water, suspension was withdrawn and filtered through a Millipore filter (pore size was $0.22\ \mu\text{m}$) at each time interval for ICP-OES analysis on an Optima 2000 (Perkin–Elmer Co.). All experiments were repeated three times.

3. Results and discussion

3.1. Bactericidal activity of CeO_2 and Ag/CeO_2

For comparison purposes, CeO_2 , 1 wt.% Ag/CeO_2 , and 2 wt.% Ag/CeO_2 were prepared. The actual contents of Ag in Ag/CeO_2 products were close to the prospective contents in the preparation process (Table 1). The bactericidal activities of CeO_2 , 1 wt.% Ag/CeO_2 , and 2 wt.% Ag/CeO_2 are shown in Fig. 1. Among the three CeO_2 shapes, bactericidal activity was in the order of CeO_2 nanocubes $\approx$ nanorods $>$ nanoparticles. Bactericidal activity was significantly improved after a small amount of silver was loaded. In general terms, bactericidal activity increased with the increase in the amount of silver. There was a 4-log decrease in *E. coli* survival number after treatment with 2 wt.% Ag/CeO_2 for 120 min. Bactericidal activity followed the order of Ag/CeO_2 nanocubes $\approx$ Ag/CeO_2 nanoparticles $>$ Ag/CeO_2 nanorods, which might be due to different surface properties and different interactions between Ag and CeO_2 support.

3.2. Characterization and effects of shape, crystalline phase and oxidation ability of CeO_2 and Ag/CeO_2 in the bactericidal process

To understand the elementary information of the catalysts, the nanomaterials were characterized by TEM and XRD. Fig. 2 shows the TEM images of CeO_2 with different shapes. The well-shaped morphologies indicated that CeO_2 nanorods, nanocubes, and nanoparticles were successfully prepared. The average diameter and length of the CeO_2 nanorods were 11 and 130 nm, respectively. The average size of the CeO_2 nanocubes was about 13 nm and CeO_2 nanoparticles had an average diameter of about 16 nm. The XRD patterns of the CeO_2 nanomaterials are shown in Fig. 3. Typical diffraction peaks of ceria fluorite structure (JCPDS 34-0394) were observed for all samples. XRD patterns of 1 wt.% and 2 wt.% Ag/CeO_2 (not shown) exhibited no obvious changes compared to those of CeO_2 . Further, after 1 wt.% and 2 wt.% Ag loaded, Ag crystalline phase was not observed in XRD patterns, which indicates that Ag showed amorphous phase or Ag crystalline size was smaller than the detection limit.

To clarify the origin of different bactericidal activities for differently shaped CeO_2 , HRTEM was carried out to examine the preferentially exposed crystal facets. Fig. 4(a) shows a HRTEM image of CeO_2 nanorods. When viewed along the $[1\ 1\ 0]$ direction, the lattice spacing of the fringes with a plane-intersecting angle of 54.7° to the elongation direction of the nanorod was 0.274 nm, which corresponded to the (200) crystal plane. Based on the interplanar spacings and plane-intersecting angles, the (200) plane and (111) side plane of the nanorods were identified, showing that CeO_2 nanorods preferred to expose the (111) and (100) planes.

Table 1

Ag content of Ag/CeO_2 product and concentration of eluted Ag^+ from different Ag/CeO_2 in deionized water.

Ag/ CeO_2 (100 mg/L)	Ag content of Ag/ CeO_2 product (wt.%)	Concentration of eluted Ag^+ (mg/L)	
		60 min	120 min
1 wt.% Ag/ CeO_2 nanorods	1.05	0.022	0.030
1 wt.% Ag/ CeO_2 nanocubes	0.99	0.066	0.074
1 wt.% Ag/ CeO_2 nanoparticles	1.08	0.291	0.327
2 wt.% Ag/ CeO_2 nanorods	1.85	0.089	0.088
2 wt.% Ag/ CeO_2 nanocubes	1.98	0.245	0.197
2 wt.% Ag/ CeO_2 nanoparticles	1.87	0.317	0.404

Fig. 1. Bactericidal activities of CeO_2 and Ag/CeO_2 against *E. coli*. Sample concentration: 100 mg/L.

The HRTEM image in Fig. 4(b) revealed that the clear (200) and (220) lattice fringes were observed with interplanar spacing of 0.274 and 0.189 nm, respectively, implying that the CeO_2 nanocubes were enclosed by the (200) planes [28]. Based on the above analysis, six side planes of the nanocubes were defined as (100) planes. A HRTEM image of the CeO_2 nanoparticles is shown in Fig. 4(c). When viewed along the $[1\ 1\ 0]$ direction, the interplanar spacing of 0.314 nm indicated the dominant presence of a (111) plane. There are three low-index planes in the ceria fluorite cubic structure, namely the very stable (111) plane, the less stable (110) plane and (100) plane [29,30]. It is also reported that less energy is required to form oxygen vacancies on (110) and (100) than on (111) plane [31]. Therefore, the energy required to create oxygen vacancies on the planes is related to their

Fig. 2. TEM images of CeO₂ (a) nanorods, (b) nanocubes, and (c) nanoparticles.

stabilities. The stability of the (111) plane is greater than that of the (100) and (110) plane, and thus it is inherently less reactive and not easier to form oxygen vacancies. CeO₂ nanocubes with exposed reactive (100) planes and CeO₂ nanorods with exposed active (100) and (111) planes resulted in much higher bactericidal activity than nanoparticles since nanoparticles favored the exposure of a large proportion of stable (111) plane on the surface.

We used 2 wt.% Ag/CeO₂ catalysts with high bactericidal activity as a typical target to study why different bactericidal activities were induced by shapes and bactericidal mechanism of Ag loaded catalysts in the following sections. Fig. 5 exhibits the HRTEM images and EDS spectra of 2 wt.% Ag/CeO₂. Silver could be found from EDS spectra which confirmed the existence of silver. However, Ag was not easily distinguished from CeO₂ in HRTEM image since the contrast between Ag and Ce is low. Furthermore, visible lattice fringes in Fig. 5 were measured and should be attributed to CeO₂ according to the analysis of lattice spacings based on the analysis in Fig. 4.

Fig. 6 shows the H₂-TPR profiles of CeO₂ and Ag/CeO₂ nanomaterials. All CeO₂ samples exhibited a broad reduction peak at 400–600 °C, which was the hydrogen consumed peak of surface oxygen [18,25]. The more intense low-temperature reduction peaks of the CeO₂ nanorods and nanocubes indicated that a higher amount of hydrogen was consumed, i.e. oxidation abilities were higher than that of nanoparticles. This observation was consistent with the trend of bactericidal activity. To investigate the reason for the obvious improvement in CeO₂ bactericidal activity of the supported silver catalysts, H₂-TPR of Ag/CeO₂ was also performed. The results showed a low-temperature reduction peak at 100–250 °C after silver addition, which was attributed to the reduction in surface oxygen and silver species [25]. The reduction peak shift to a lower temperature indicated that the oxidation ability of Ag/CeO₂ largely increased compared with CeO₂, which might be helpful for the

increase in Ag/CeO₂ bactericidal activity. However, the reduction peak temperature of the Ag/CeO₂ nanorods and the intensity of the low-temperature reduction peak of the Ag/CeO₂ nanoparticles were the lowest, which was not consistent with the order of bactericidal activity. The H₂-TPR results reflected that the oxidation ability of solid catalysts might be somewhat different from the oxidation activity of catalysts placed in aqueous solution at room temperature. Furthermore, the bactericidal process in water was complex. Therefore, examination of Ag⁺ elution and ROS formation was performed to elucidate the discrimination of bactericidal activity and the mechanism of *E. coli* death.

3.3. Effect of Ag⁺ in the bactericidal process

When using silver-loaded catalysts, Ag⁺ elution cannot be avoided under aqueous conditions. It is generally accepted that Ag⁺ at high concentrations exhibits bactericidal activity [32]. The concentrations of eluted Ag⁺ measured during duration time in water with vigorous stirring are shown in Table 1. The concentration of Ag⁺ eluted from 1 wt.% Ag/CeO₂ nanorods was 0.030 mg/L within 60 min. As silver increased to 2 wt.%, the concentration of Ag⁺ eluted from Ag/CeO₂ increased. The concentration of Ag⁺ eluted from Ag/CeO₂ nanorods was much less than that eluted from nanoparticles, indicating that Ag/CeO₂ nanorods largely reduced the speed of silver elution, while the concentration of Ag⁺ eluted from nanocubes was found to be moderate. Recent determination of metal adsorption energies from calorimetric measurements found strong interaction and bonding between Ag and CeO₂ [33]. Consequently, the origin of different concentrations of Ag⁺ eluted from variously shaped CeO₂ with different exposed crystal facets might be associated with the bond stability offered by the strength of the chemical bonds between Ag and the underlying oxide surface. The strong chemical bonding of Ag to CeO₂ might lead to the lower concentration of Ag⁺ elution. As shown in Fig. 1, 0.5 mg/L Ag⁺ induced only a 2 log decrease in the survival cells of *E. coli* in 120 min, while the concentrations of Ag⁺ eluted from Ag/CeO₂ catalysts were less than 0.5 mg/L, which meant that the toxicity of Ag⁺ did not play an important role in the bactericidal process since a 5 log decrease was achieved for 2 wt.% Ag/CeO₂. Of course, the highest concentration of Ag⁺ eluted from the Ag/CeO₂ nanoparticles inevitably had a minor contribution to the inactivation of *E. coli*, which explained why Ag/CeO₂ nanoparticles exhibited high bactericidal ability despite their low oxidation ability. On the surface of solid CeO₂, •O₂[−] has been detected using ESR [17]. It was expected that ROS might form inside cells or on the surface of catalysts existing in *E. coli* suspension, promoted by Ag particles and CeO₂, and might play a key role in inactivation of *E. coli*.

3.4. Effect of ROS in the bactericidal process

Because •OH and •O₂[−] are very unstable in water, 5, 5-dimethyl-1-pyrroline-*N*-oxide (DMPO) is usually used as the spin-trapping reagent

Fig. 3. XRD patterns of CeO₂.

Fig. 4. HRTEM images of CeO₂ (a) nanorods, (b) nanocubes, (c) nanoparticles.

to determine $\bullet\text{OH}$ and $\bullet\text{O}_2^-$ radicals. Fig. 7 illustrates the ESR spectra of DMPO- $\text{OH}^\bullet$ spin adduct and DMPO- $\text{O}_2^{\bullet-}$ spin adduct measured immediately after the mixing of the catalysts with DMPO solution at room temperature. As shown in Fig. 7(a), the four characteristic peaks of the DMPO- $\text{OH}^\bullet$ species, a 1:2:2:1 quartet pattern, were clearly observed

after the addition of the catalysts. Similarly, the six characteristic peaks of DMPO- $\text{O}_2^{\bullet-}$ adducts were also observed in methanol solution after the addition of the catalysts, as shown in Fig. 7(b). Even though $\bullet\text{OH}$ and $\bullet\text{O}_2^-$ were both produced on the catalyst surface, the signals of DMPO- $\text{OH}^\bullet$ were obviously stronger than those of DMPO- $\text{O}_2^{\bullet-}$,

Fig. 5. HRTEM images of 2 wt.% Ag/CeO₂ (a) nanorods, (b) nanocubes, (c) nanoparticles and EDS spectra of 2 wt.% Ag/CeO₂ (d) nanorods, (e) nanocubes, (f) nanoparticles. The circle ozone was the scan ozone of EDS.

Fig. 6. H_2 -TPR profiles of CeO_2 and Ag/CeO_2 .

indicating that $\bullet OH$ might be primarily responsible for the catalytic oxidation of *E. coli* cells. The signal intensities of $\bullet OH$ formed on the surface of Ag/CeO_2 catalysts were obviously larger than those formed on the surface of CeO_2 , which were consistent with the order of bactericidal activities. Thus, direct evidence of $\bullet OH$ and $\bullet O_2^-$ formation on the surface of CeO_2 and Ag/CeO_2 provided a strong indication that the catalyst effectively activated the adsorbed oxygen to produce a series of active oxygen species, which played important roles in the inactivation of *E. coli*. Furthermore, the formation of H_2O_2 was also confirmed by the addition of 286 units/mL catalase as a scavenger of H_2O_2 (Fig. 8). After the addition of catalase, the bactericidal activities of the Ag/CeO_2 nanorods were inhibited dramatically. Similar phenomena were also observed for other

Fig. 7. DMPO spin-trapping ESR spectra recorded at ambient temperature (a) in aqueous dispersion (for $DMPO-OH^\bullet$ adduct) and (b) in methanol dispersion (for $DMPO-O_2^{\bullet-}$ adduct).

Fig. 8. Effect of catalase against H_2O_2 on the bactericidal activity of Ag/CeO_2 nanorods.

shapes of Ag/CeO_2 . These results indicate that the formation of H_2O_2 also significantly contributed to the bactericidal process.

To understand the formation mechanism of extracellular ROS, the states of highly dispersed Ag species and CeO_2 support were determined through $Ag3d$ and $Ce3d$ binding energy analysis. Fig. 9 shows the XPS spectra of $Ag3d$ and $Ce3d$. As previously reported, the binding energy of the $Ag3d_{5/2}$ peaks of Ag^0 and Ag_2O are located at 368.3 and 367.8 eV, respectively [34]. Therefore, Ag^0 and Ag^+ co-existed in Ag/CeO_2 catalysts. Interestingly, the ratio of Ag to Ce on the catalyst surfaces showed significant distinction with different shapes of CeO_2 supports, which implied that Ag dispersion was different on the surface of three types of Ag/CeO_2 (Table 2). The ratio of Ag to Ce on the surface of 2 wt.% Ag/CeO_2 nanocubes was the highest, while the ratio of Ag to Ce on the surface of 2 wt.% Ag/CeO_2 nanoparticles was the

Fig. 9. (a) $Ag3d$ and (b) $Ce3d$ XPS spectra of Ag/CeO_2 .

Table 2Atomic ratio of Ag and Ce from Ag3d and Ce3d XPS spectra on the surface of Ag/CeO₂.

	At%	
	Ag3d	Ce3d
2 wt.% Ag/CeO ₂ nanorods	6.12	93.88
2 wt.% Ag/CeO ₂ nanocubes	8.40	91.60
2 wt.% Ag/CeO ₂ nanoparticles	3.35	96.65

lowest. These results indicate that among the three types of catalysts, silver had the highest degree dispersion on the surface of CeO₂ nanocubes and silver might form large clusters or particles on the surface of CeO₂ nanoparticles. The higher dispersion was helpful for the formation of •OH radical (Fig. 7(a)). The XPS data for the Ce3d region from Ag/CeO₂ are shown in Fig. 9(b). Six peaks corresponding to three pairs of spin-orbit split doublets were identified in the spectrum, which were associated with Ce⁴⁺. These peaks were labeled using conventional notations (U, U', U'', V, V', and V'') as described previously [18]. U and V referred to 3d_{3/2} and 3d_{5/2}, respectively. Furthermore, U' and V' were associated with Ce³⁺. By fitting the Ce3d XPS spectra based on the reference spectra, Ce existed in the catalysts as Ce³⁺ and Ce⁴⁺. The appearance of Ce³⁺ indicates the formation of surface oxygen vacancies on CeO₂ [35]. In this study, the formation of •O₂⁻ and H₂O₂ in the solution was thought to be dependent on the ability of CeO₂ to activate/store oxygen and transform/release oxygen species controlled by the distribution of oxygen vacancies as the most relevant surface defects, resulting from the redox cycle of Ce³⁺/Ce⁴⁺ [36–38]. Moreover, when Ag was dispersed as metal particles on some oxides, the Ag particle surfaces might have sufficient defects for dissociative chemisorption of oxygen [20,39]. Therefore, it was believed that the redox cycle of Ag⁺/Ag⁰ and Ce³⁺/Ce⁴⁺ co-maintained the catalytic process of ROS formation. Thus, we supposed that dissolved O₂ in water was first chemisorbed on the oxygen vacancy site adjacent to Ce³⁺ to yield reactive oxygen species such as •O₂⁻, •O₂²⁻ or H₂O₂, accompanied by the production of Ce⁴⁺. Because Ag⁺ could oxidate OH⁻ to H₂O₂, Ag⁰ could catalyze the oxidizing action of H₂O₂ by forming •OH through a Fenton-like reaction. The formation of ROS was proposed as follows.

Thus, extracellular reactive oxygen species migrated to the surface of *E. coli* and oxidized cells, leading to cell death.

To examine the effect of ROS induced by Ag/CeO₂ on *E. coli* cells in vivo, DCFH-DA was used as an intracellular ROS-indicator for the Ag/CeO₂ treated cells to measure the generation of intracellular ROS [27]. Fig. 10 shows that *E. coli* cells became DCF positive after Ag/CeO₂ treatment, indicating that intracellular ROS were generated and participated in the Ag/CeO₂-induced cell death. For comparison, relative fluorescence intensity (i.e. relative ROS level) is presented in Fig. 11. The cells treated with Ag/CeO₂ nanorods displayed the highest relative ROS level. In contrast, the cells treated with Ag/CeO₂ nanoparticles had the lowest relative ROS level. The sequence of intracellular ROS amount corresponded with that of extracellular ROS amount produced through the Ag/CeO₂ catalysts. The cells treated with 0.5 mg/L Ag⁺ had lower relative ROS levels than cells treated with Ag/CeO₂. These results suggest that although Ag⁺ also aided the generation of intracellular ROS through respiratory enzymes [40], extracellular ROS played a more important role in the production of intracellular ROS and cell inactivation. Intracellular ROS, induced by extracellular ROS and Ag⁺, was a candidate mediator for cell apoptosis and death.

Fig. 10. Photographs of fluorescence microscope of *E. coli* treated with 2 wt.% Ag/CeO₂ samples for 2 h respectively after stained with 10 μM DCFH-DA.

Fig. 11. Ag/CeO₂ induced ROS production in *E. coli* cells. Average of fluorescence intensities in each group was expressed. The cells with DCFH-DA labeling served as negative control, and H₂O₂ was used as positive control. 0.5 mg/L Ag⁺ was used as another control. Sample concentration, 100 mg/L; initial bacterial concentration, 1 × 10⁷ CFU/mL.

Fig. 12. TEM images of (a) untreated *E. coli* cells and (b) *E. coli* cells treated with 2 wt.% Ag/CeO₂ nanorods for 0.5 h, (c) 1 h, (d) 3 h.

3.5. Morphological and structural change of cell wall and cell membrane

Fig. 12 shows the TEM images of *E. coli* cells before and after treatment with 2 wt.% Ag/CeO₂ nanoparticles. As shown, the catalyst congregated on the surface of the cell and the shape of cell was not obviously changed after 0.5 h treatment (Fig. 12(b)), compared with untreated cells (Fig. 12(a)). After a 1 h treatment, the cell wall was damaged and cellular leakage appeared (Fig. 12(c)). With prolonged treatment, cells were significantly damaged, leading to a leakage of intracellular constituents (Fig. 12(d)). These phenomena were different from the effect of one-fold Ag⁺ [16], and it could be deduced that ROS were more active in destroying *E. coli* cells. Previous research reported that large electron-dense granules were observed around the cell wall after treatment with 0.5 mg/L Ag⁺ [16]. Experimental results also revealed that the zeta potential of Ag/CeO₂ was positive, with a value of 11 mV and a concentration of 100 mg/L, whereas the surface of *E. coli* was negatively charged in neutral solution. Therefore, electrostatic attraction between the catalyst and *E. coli* might be the reason for the easy absorbance of Ag/CeO₂ on the surface of cells, which might be helpful for the catalytic inactivation of *E. coli* by ROS.

The membrane integrity of cells was reflected by the influx of membrane-impermeable fluorescent PI. Fig. 13 shows that cells treated with Ag/CeO₂ were PI positive. The number of PI positive cells increased with the increase of bactericidal activity. Thus, the death of cells induced by Ag/CeO₂ involved the disruption of membrane integrity through the generation of intracellular ROS.

4. Conclusion

In conclusion, difference in the exposed crystal planes as well as oxidation ability among CeO₂ nanocubes, nanorods and nanoparticles resulted in much higher bactericidal activities of CeO₂

nanocubes and nanorods than that of nanoparticles. When CeO₂ was loaded with a small amount of Ag, the bactericidal activities largely increased with the increase of oxidation ability. Extracellular ROS with strong oxidative capabilities, such as •OH and •O₂⁻, were successfully detected by ESR at room temperature, which provided direct proof for the catalytic inactivation of *E. coli* cells by CeO₂ based catalysts through the activation of molecular oxygen without extra light or electric power input. The formation of H₂O₂ was confirmed by the addition of catalase, which also played an important role in the inactivation of *E. coli*. Furthermore, the toxicity of Ag⁺ eluted from Ag/CeO₂ also provided a minor contribution. In all, extracellular ROS and Ag⁺ induced the production of intracellular ROS, leading to the disruption of the cell wall and cell membrane, and subsequent cell death. These results indicated that catalytic oxidation was the essential mechanism in the bactericidal process.

Abbreviations

ROS	reactive oxygen species
ICP-OES	inductively coupled plasma optical emission spectrometer
XRD	X-ray diffraction
HRTEM	high-resolution transmission electron microscope
EDS	energy dispersive spectroscopy
XPS	X-ray photoelectron spectroscopy
ESR	electron spin resonance
H ₂ -TPR	temperature-programmed reduction of H ₂
LB	lactose broth
CFU/mL	colony forming units per milliliter
DCFH-DA	2',7'-dichlorofluorescein- diacetate
PBS	phosphate-buffered saline
TEM	transmission electron microscopy
PI	propidium iodide

Fig. 13. Ag/CeO₂ induced membranes disruption of *E. coli* cells. The photographs of fluorescence microscope of *E. coli* treated with 2 wt.% Ag/CeO₂ samples for 2 h respectively, where dead cells were positive for PI (red). Sample concentration, 100 mg/L; initial bacterial concentration, 1.5×10^7 CFU/mL.

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